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Broad spectrum anthelmintic potential of *Cassia* plants

Suman Kundu, Saptarshi Roy, Larisha Mawkhleing Lyndem*

Parasitology Research Laboratory, Department of Zoology, Visva Bharati, Santiniketan-731235, West Bengal, India

PEER REVIEW

Peer reviewer

Dr. WABO Pone Josué, University of Dschang, Department of Animal Biology, PO Box 067 Dschang, Cameroon.
Tel: +237 75 39 03 18/99521863
E-mail: waboponejosue@yahoo.fr

Comments

This is a good study in which the authors evaluated the anthelmintic effect of *C. alata*, *C. angustifolia*, and *C. occidentalis* for the control of gastro-intestinal parasitism. The three different leaf extracts were compared for their efficacy and were found to have different levels of efficacy depending on the parasite species. The results are interesting and suggest that anthelmintic substances are present in leaves of three plant species.
Details on Page S440

ABSTRACT

Objective: To study the *in vitro* anthelmintic efficacy of *Cassia alata* (*C. alata*), *Cassia angustifolia* (*C. angustifolia*) and *Cassia occidentalis* (*C. occidentalis*).
Methods: Crude ethanol extract from leaves of the three plants were prepared in rotary evaporator and different concentrations (10, 20 and 40 mg/mL) of leaf extracts were used for treatment on different representatives of helminthes (*Heterakis gallinarum*, *Raillietina tetragona* and *Catatropis* sp.) from domestic fowl (*Gallus gallus domesticus*). Loss of motility and death were monitored frequently.
Results: *C. alata* showed early paralysis in all worms treated followed by *C. angustifolia*. *C. occidentalis* in combination with *C. alata* together caused early paralysis in all treated worms than the combination of *C. alata* with *C. angustfolia*. While *Heterakis gallinarum* in control survived for (81.33±2.07) h, treated worms lost their motility at (5.71±0.10) h, (6.60±0.86) h and (13.95 ±0.43) h with *C. angustifolia*, C. alata and *C. occidentalis* respectively at a concentration of 40 mg/mL which showed better efficacy than albendazole. *Catatropis* sp. survival period was (26.49±1.38) h in control, but with plant treatment, it lost its motility in just (0.57±0.08) h, (1.00±0.12) h and (1.47 ±0.40) h at 40 mg/mL concentration of *C. alata*, *C. angustifolia* and *C. occidentalis* respectively. *Raillietina tetragona* on the other hand became paralysed at (1.68±0.27) h, (2.95±0.29) h and (4.13 ±0.31) h with above concentrations treated with three plants respectively, however in control it survived up to (81.93±4.71) h.
Conclusions: This present study indicated broad spectrum vermifugal activity of all plants tested.

KEYWORDS
Anthelmintic, *Cassia*, Parasites, Extracts, Paralysis, Vermifugal

1. Introduction

Helminth parasites problem is a major limiting factor in livestock production. Control of helminth infection in domestic animals is widely based on anthelmintic drugs. Its prolonged use has led to the additional problem of emergence of anthelmintic resistant in livestock which has become a constant concern. Dependence of control programs on a limited number of compounds makes drug resistance an even greater concern and careful management of those available is imperative. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore such plants should be investigated to better understand its safety and efficacy[1]. The origin of many effective drugs is found in traditional medicine practices and has made several researchers to undertake studies for evaluating folklore medicinal plants on their proclaimed anthelmintic efficacy[2-4]. Use of medicinal plants and studies on the chemistry and pharmacology of natural products have grown considerably in the second half of 20th century[5,6]. Medicinal value of plants is attributed to only few active constituents that produce a definite physiological effect. Alkaloids, flavonoids,

*Corresponding author: Larisha Mawkhlieng Lyndem, Department of Zoology, Visva Bharati, Santiniketan-731235, West Bengal, India.
Tel: +919434759390
E-mail: lyndemlarisha@hotmail.com

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tannins and phenolic compounds form a major group of such bioactive constituents[7]. Plants and herbs have attained a significant role not only as therapeutic agent but also as health maintaining agent.

Cassia plants are widely distribute in India and West-Bengal in particular and its many species are having medicinal activities. *Cassia alata* (*C. alata*) has been recognized for centuries in traditional medicine for its activities such as antimicrobial[8], antifungal[9], purgative[10], anti-inflammatory[9,11], analgesic[12], antitumor[13], and hypoglycemic[9]. *Cassia angustifolia* Vahl. (*C. angustifolia*) is known for its purgative properties and for regularization of bowel movements[14] while *Cassia occidentalis* Linn. (*C. occidentalis*) have been traditionally used for treatment of constipation, skin diseases, as laxative and diuretic[15]. It is also used for treatment of fever, menstrual problems, tuberculosis, diuretic, anemic, liver complaints, and as a tonic for general weakness and illness. Decoction of leaves of *C. angustifolia* and *C. alata* is widely practiced in India as compared to other parts of the plant. In addition, their leaves have been listed as a treatment for gastrointestinal worms in medicinal plant resources[14].

Furthermore, these plants have attracted the attention of many scientists because of the presence of flavonoid compounds, anthraquinones and phenols. Kundu and Lyndem[16] reported anthelmintic activity of the above three *Cassia* plants on *Raillietina tetragona* (*R. tetragona*) as well as *C. alata* on *Hymenolepis diminuta*[17]. Moreover like commercial drugs themselves, majority of anthelmintic plants are helminth specific, showing activity against a particular species or group of parasites[18]. In order to authenticate their broad anthelmintic spectrum, it is necessary to investigate the effect of all three *Cassia* species on common helminthes of domestic fowl (*Gallus gallus domesticus*).

2. Materials and methods

2.1. Preparation of plant extracts

Fresh young leaves of *C. angustifolia*, *C. alata* and *C. occidentalis* were collected, weighed and washed with deionized water, and oven-dried at 50 °C. About 150 g of powdered leaves were extracted with 1 L of ethanol (90%) in a Soxhlet apparatus for 7-8 h. The final crude extracts were recovered using a rotary evaporator and stored at 4 °C until further use.

2.2. Chemicals and drugs

All chemicals used were of standard analytical grade, obtained from Merck (USA). Ethanol was supplied by Bengal Chemicals (Kolkata, India). Two reference drugs praziquantel with trade name Distocide (composed of 600 mg praziquantel) is a product of Chandrabhagat Pharma Pvt. Ltd (Mumbai, India) and albendazole with trade name Zentel (composed of 400 mg albendazole) is a product of Glaxo SmithKline Pharmaceuticals Ltd (Mumbai, India).

2.3. Experimental design

Helminth representatives *viz. Heterakis gallinarum* (nematoda) (*H. gallinarum*), *Catatropis* sp. (trematoda) and *R. tetragona* (cestoda) were collected from intestine of freshly slaughtered domestic fowl. Live worms were treated *in vitro* with three leaf extracts at various concentrations (10, 20 and 40 mg/mL, in 0.9% phosphate-buffered saline (pH 7.4). While 5, 10 and 20 mg/mL of albendazole was used as a reference drug for *H. gallinarum*, praziquantel (0.001, 0.0025 and 0.005 mg/mL) was tested for the other two worms. Control worms were maintained in phosphate-buffered saline with 1% dimethylsulfoxide at (37±1) °C. Combinations of plant extracts in ratio 1:1 (*C. alata*+*C. occidentalis*, *C. alata*+*C. angustifolia*, *C. occidentalis*+*C. angustifolia*) were also tested on fresh worms at (37±1) °C to observe any synergistic or additive effect.

2.4. Statistical analysis

Data were represented as mean±SD for each group (n=6). For determining statistical significance, SD and analysis of variance (ANOVA) at 5% level significance were employed; P<0.005 was considered significant.

3. Results

However, since each tested worms are different, it is therefore convenient to compare different leaf extracts of *Cassia* plants on a particular parasite.

3.1. Single plant extract treatment

For *H. gallinarum*, *C. angustifolia* showed early paralysis at (16.12±3.36) h compared to other two plants at 10 mg/mL, but at 20 mg/mL, both *C. alata* and *C. angustifolia* took almost equal time [(11.57±1.43) h and (11.79±3.19) h] to paralyze the worm. At 40 mg/mL paralysis occurred early with *C. angustifolia* [(5.71±0.1) h] followed by *C. alata* [(6.6±0.86) h] and *C. occidentalis* [(13.95±0.43) h] (Table 1). Time of mortality of parasites was reduced to (45.92±1.07) h with *C. alata* treatment at 10 mg/mL which significantly differed from *C. occidentalis* [(52.65±3.73) h] and *C. angustifolia* [(53.75±2.42) h]. Same trend of

Table 1

Effects of crude ethanolic extracts of different *Cassia* species on three helminths of domestic fowl.

Worm types	Concentration (mg/mL)	*C. alata*		*C. angustifolia*		*C. occidentalis*	
		PT (h)	TM (h)	PT (h)	TM (h)	PT (h)	TM (h)
H. gallinarum	10	24.93±1.67	45.92±1.07	16.12±3.36	53.75±2.42	29.92±3.35	52.65±3.73
	20	11.57±1.43	27.90±1.20	11.79±3.19	32.70±1.26	22.86±3.61	37.69±4.26
	40	6.60±0.86	19.42±0.66	5.71±0.10	17.86±0.18	13.95±0.43	29.76±4.70
Catatropis sp.	10	2.83±0.08	7.33±0.14	3.74±0.17	6.70±0.17	6.42±0.53	12.37±0.88
	20	2.32±0.15	4.27±0.10	1.59±0.10	3.67±0.08	3.18±0.53	9.24±0.17
	40	0.57±0.08	2.78±0.31	1.00±0.12	3.06±0.09	1.47±0.40	4.71±0.14
R. tetragona	10	3.10±0.14	15.88±0.46	14.16±0.03	31.14±0.68	32.75±0.15	48.06±0.11
	20	2.89±0.22	13.10±0.37	5.39±0.46	21.15±0.63	9.31±0.18	26.96±0.24
	40	1.68±0.27	8.55±0.32	2.95±0.29	18.52±0.09	4.13±0.31	22.40±1.21

Values are expressed as mean±SD. Survivability of parasites in control medium: *H. gallinarum*=(81.33±2.07) h; *R. tetragona*=(81.93±4.71) h and *Catatropis* sp.=(26.49±1.38) h. PT: time of paralysis; TM: time of mortality.

observation were seen at higher concentration. However parasite survived up to (81.33±2.07) h in control.

With albendazole, both paralysis and time of mortality showed significantly longer time [(45.17±1.84) h and)37.75 ±1.47) h] compared to all three plant extracts tested, at 10 and 20 mg/mL consecutively (Table 2).

Table 2

Effect of albendazole on *H. gallinarum*.

Worm	Concentration (mg/mL)	PT (h)	TM (h)
H. gallinarum	5	55.45±1.23	72.60±1.85
	10	45.17±1.84	64.99±1.32
	20	37.75±1.47	57.56±1.91

Values are expressed as mean±SD. Survivability of parasites in control medium: (81.33±2.07) h. PT: time of paralysis; TM: time of mortality.

For *R. tetragona*, each plant extract has dose-dependent efficacy (Table 1). In all concentrations, *C. alata* showed significantly less time to paralyse [(3.1±0.14) h, (2.89±0.22) h and (1.68±0.27) h], followed by *C. angustifolia* [(14.16± 0.03) h, (5.39±0.46) h and (2.95±0.29) h] and *C. occidentalis* [(32.75±0.15) h, (9.31±0.18) h and (4.13±0.31) h]. Time of mortality also showed a similar pattern with *C. alata* taking a shorter time and *C. occidentalis*, a longer time but in control the parasite survived up to (81.93± 4.71) h (Table 1).

Praziquantel treatment on parasites showed both paralytic and mortality time were comparatively short as compared with plant treated parasites even at low concentrations (Table 3).

Table 3

Effect of praziquantel on *R. tetragona* and *Catatropis* sp.

Worm	Concentration (mg/ml)	PT (h)	TM (h)
R. tetragona	0.001 0	1.27±0.37	21.37±1.87
	0.002 5	0.76±0.13	19.14±1.18
	0.005 0	0.17±0.01	17.07±0.31
Catatropis sp.	0.001 0	2.50±0.25	28.86±0.37
	0.002 5	1.09±0.20	21.00±0.91
	0.005 0	0.30±0.04	13.09±0.37

Values are expressed as mean±SD. Survivability of parasites in control medium: *R. tetragona*=(81.93±4.71) h and *Catatropis* sp.=(26.49±1.38) h. PT: time of paralysis; TM: time of mortality.

For *Catatropis* sp. at 10 mg/mL, *C. alata* caused early paralysis [(2.83±0.08) h] amongst the three plants, followed by *C. angustifolia* [(3.74±0.17) h] and *C. occidentalis* [(6.42 ±0.53) h]. While at 40 mg/mL, there was slight decline in the level of significance in all tested plant extracts when compared between paralysis time and time of mortality. However, control parasite survived up to (26.49±1.38) h (Table 1).

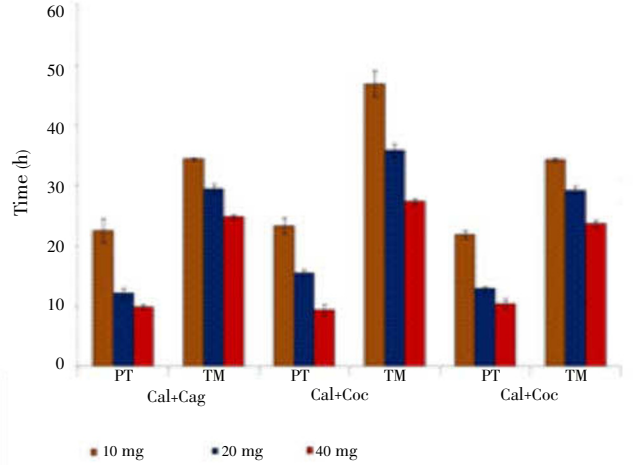

Figure 1. Effect of combinations of *Cassia* plant extracts on *H. galinarum*. Cal: *C. alata*; Cag: *C. angustifolia*; Coc: *C. occidentalis*.

3.2. Treatment with combination of two plant extracts

For *H. gallinarum*, a combination of *C. angustifolia*+*C. occidentalis* was observed to cause early paralysis [(21.95 ±0.64) h] followed by *C. alata*+*C. angustifolia* [(22.53±1.97) h] at 10 mg/mL. However at 40 mg/mL, there was a decline in the level of significance of *C. alata*+*C. occidentalis* and *C. alata*+*C. angustifolia*. While time of mortality was shown to occur at (23.69±0.53) h by *C. angustifolia*+*C. occidentalis*, it took about (27.39±0.51) h in case of *C. alata*+*C. occidentalis* (Figure 1).

In *R. tetragona* at 40 mg/mL, paralysis showed no significantly different in all combinations, but the time of mortality showed more significantly different in *C.*

alata+*C. angustifolia* with *C. angustifolia*+*C. occidentalis* (Figure 2).

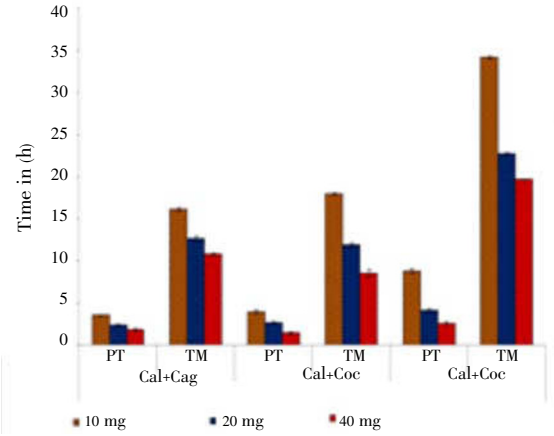

Figure 2. Effect of combinations of *Cassia* plant extracts on *R. tetragona*. Cal: *C. alata*; Cag: *C. angustifolia*; Coc: *C. occidentalis*.

For *Catatropis sp*., C. alata + *C. angustifolia* having more effects in causing early paralysis and mortality in all concentrations followed by C. alata + *C. occidentalis* and *C. angustifolia* + *C. occidentalis* (Figure 3).

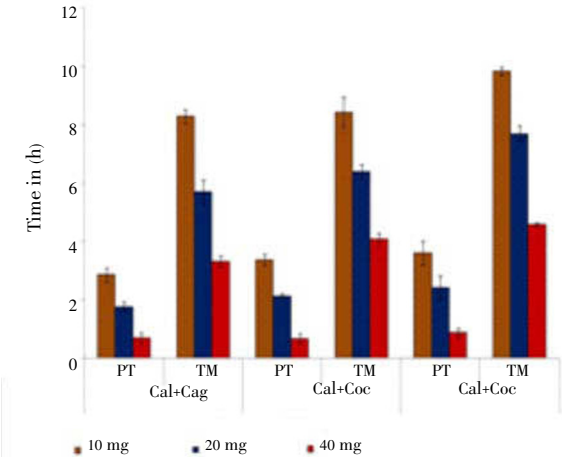

Figure 3. Effect of combinations of *Cassia* plant extracts on *Catatropis* sp. Cal: *C. alata*; Cag: *C. angustifolia*; Coc: *C. occidentalis*.

4. Discussion

The present investigation showed paralysis preceded mortality very significantly and paralysis of worms occurred for a very short interval following exposures of parasites to these plant extracts. Similar *in vitro* studies were also observed in ethanol extract of *Artemesia cina* on *Moniezia expansa*[19]; *Artemesia annua*, *Artemesia absinthium* and *Fumaria officinalis* on *Schistosoma mansoni* and *Fasciola hepatica*[20]; *Cassia alata* on *Hymenolepis diminuta*[17].

C. angustifolia showed more effect on *H. gallinarum* compared to other two plants. For *R. tetragona* and *Catatropis* sp., it was *C. alata* that showed more effects followed by *C. angustifolia* in causing immobility. Though varying rate of efficacy results, these variations in efficacy may be due to different chemical constituents between plant individuals resulting from differences in solubility in ethanol[21]. The effects responsible for anthelmintic activity were similar; all concentrations used have caused moderate level of effects on the motility of tested worms. This inactiveness could lead to expulsion of paralysed worms from the body[22].

Current study inhibited motility at low concentration, increasing the concentration of the plant extracts resulted in early paralysis, thus indicating dose-dependent efficacy of the test materials. Similar observations were also recorded with leaf extract of *Stephania glabra* and *Trichosanthus multiloba* on *Ancylostoma ceylanicum*[23], *Alocasia indica* on *Pheretima posthuma*[24], *Acacia oxyphylla* on *Raillietina echinobothrida*[25], extracts from coconut, onion, garlic, fig on different cestodes and trematodes[26,27].

Albendazole is known to cause paralysis of worms so that they are expelled in faeces of man and animals. Experimental plant extracts not only demonstrated this property, but they also caused early death of worms at all concentrations compared to drug. Thus findings from the current study revealed that extracts have shown promising *in vitro* anthelmintic activity. These plants are known to contain large amount of alkaloids, flavonoids, glycosides, tannins[6]. This anthelmintic activity could be attributed to these bioactive compounds jointly or separately. Classes of secondary metabolites such as alkaloids and flavonoids are considered the source of chemical components responsible for wide therapeutic activities of several medicinal plants[7]. The antimicrobial activities of *C. alata*, *C. angustifolia* and *C. occidentalis* have been ascribed mainly due to the presence of alkaloids, flavonoids, glycosides, tannins[7]. Similarly, Athanasiadou *et al*. suggested that anthelmintic activity of plant extracts on larvae and adults of gastrointestinal nematodes could be attributed to tannins' capacity to bind to proteins and could operate via several mechanisms[28]. Maqbool et al. suggested that anthelmintic activity of *Fumaria parviflora* may be due to alkaloids which have the ability to intercalate with DNA synthesis of parasites[29]. Transcuticular diffusion is a common means of entry into helminth parasites for non-nutrient and non-electrolyte substances in nematode, cestode and trematode parasites as opposed to oral ingestion[30,31]. Possible explanation for better anthelmintic activity of ethanolic extracts could

be due to easier transcuticular absorption of ethanolic extracts into body of parasites.

Our current study revealed broad anthelmintic activity of all three plants tested and their broad activities are described for the first time. Delaquis *et al*. observed naturally occurring combinations of plant compounds showed synergistic and often results in crude extracts having greater antimicrobial activity than the purified individual constituents[32].

Such plant based treatments could be made part of an integrated management plan for control of helminths in developing countries. Further studies are required to isolate and reveal the active compound contained in the crude extracts to establish the mechanism of action.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Helminthiasis has become a worldwide problem for animals and humans. The intensive use of chemical anthelmintics has led to the development of parasite resistance. To try and solve this problem, several alternative methods are studied like phytotherapy, which is used by traditional healers or by indigenous people for humans or animals.

Research frontiers

Studies are being performed in order to scientifically evaluate the traditional anthelmintic use of *C. alata*, *C. angustifolia*, *C. occidentalis* plants used in India and West-Bengal for various ailment. The efficacy of these three leaf extracts was tested against the fowl parasites *H. gallinarum*, *Catatropis* sp. and *R. tetragona*.

Related reports

The lack of effect of some extracts (the water extract here) has to be reconsidered as it was demonstrated in other studies (Raskin *et al*., 2002) so that it could vary depending on several parameters in the plant (origin, soil, age, season, *etc*.) and also the type of parasite tested.

Innovations and breakthroughs

An innovation in the paper is that several parasites of different classes were tested.

Applications

The results of this study suggest that the three extracts of the leaves of *C. alata*, *C. angustifolia* and *C. occidentalis* were active against three parasite species of fowl. Thus it is important.

Peer review

This is a good study in which the authors evaluated the anthelmintic effet of *C. alata*, *C. angustifolia*, and *C. occidentalis* for the control of gastro-intestinal parasitism. The three different leaf extracts were compared for their efficacy and were found to have different levels of efficacy depending on the parasite species. The results are interesting and suggest that anthelmintic substances are present in leaves of three plant species.

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